

Certificate of Analysis

Src

(Recombinant protein expressed in Sf21 insect cells)

Item # 23-042, 23-042-K, 23-042M

Parent Lot # D11HP001N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal c-Myc, 6His- tagged, recombinant human Src full length, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.

Purity 94% by SDS-PAGE and Coomassie blue staining. MW = 65kDa.

Formulation: 1.607mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

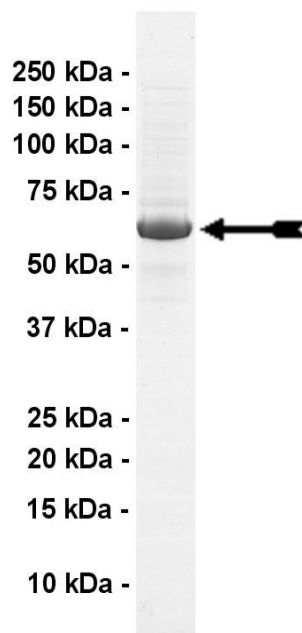
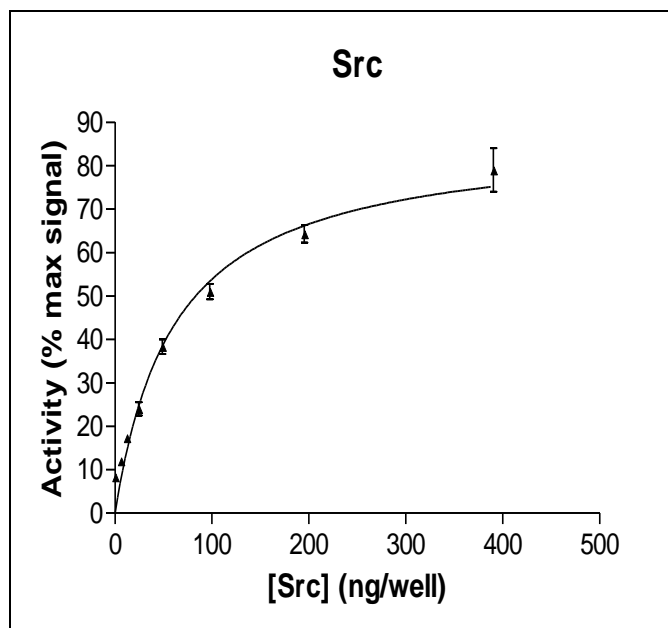
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Assay: This enzyme was titrated in a ubiquitination assay and the results normalised against the maximum signal.

Protein Identity: Confirmed identity as Src by mass spectrometry.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of Src.

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Assay Protocol

Reagents:

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|-----------------------------------|---------------------------|
| 1. UBE1, active (Item # 23-021) | 4. 1x Reaction Buffer |
| 2. UbchH4, active (Item # 23-025) | 5. Biotinylated-Ubiquitin |
| 3. c-Cbl, active (Item # 23-041) | 6. Stop Solution |

Assay Outline:

All enzymes and reagents are diluted in the 1x reaction buffer (25mM MOPS pH7.5, 0.01% Tween 20, 5mM MgCl₂).

Src is incubated with 25mM MOPS pH7.5, 0.01% Tween 20, 5mM MgCl₂, 10μM ATP, 10nM UBE1, 100nM UbchH4, 5μg/ml c-Cbl, and 2μM biotinylated-ubiquitin. The reaction is initiated with the addition of biotinylated-ubiquitin. After 30 minutes at room temperature the reaction is terminated by the addition of 25mM MOPS pH 7.5 containing 125mM EDTA, 150mM NaCl, and 0.05% Tween 20. Reaction products are separated by capture onto a microplate coated with anti-c-Myc antibody and washing with PBS containing 0.05% Tween 20. Ubiquitination of Src is measured by detection of bound ubiquitin via electrochemiluminescence.

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Src Information

<u>Protein</u>	human Src
<u>Accession number</u>	GenBank NM_198291
<u>Alternative Names</u>	Proto-oncogene c-Src, pp60c-src, SRC1

<u>Key Facts</u>	Src is a ubiquitously expressed, non-receptor tyrosine kinase that plays a pivotal role in numerous cellular processes such as proliferation, migration, and transformation. Due to its critical function in regulating signal transduction pathways, Src activity is tightly controlled through reversible phosphorylation and dephosphorylation events and down-regulation by ubiquitin-dependent degradation. Dysfunction of Src, through overexpression or increased activation has profound effects on basic cellular functions. Elevated Src expression and/or activation is evident across a wide range of solid tumour types and has been strongly implicated in the development, maintenance, progression, and metastatic spread of several human cancers, such as prostate, lung, breast, and colorectal cancer.
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<u>Related Products</u>	Item # 23-021 UBE1, active, Item # 23-025 UbchH4, active, Item # 23-041 c-Cbl,active
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Selected References

- Wheeler D. L. *et al.*, The Role of Src in Solid Tumors. *Oncologist*, 14: 667-78, 2009
- Korade-Mirnic Z. and Corey S. J. Src kinase-mediated signaling in leukocytes. *J Leukoc Biol.* 68: 603-613, 2000
- Harris K.F. *et al.*, Ubiquitin-Mediated Degradation of Active Src Tyrosine Kinase. *PNAS.* 96: 13738-13743, 1999
- Yokouchi M. *et al.*, Src-Catalyzed Phosphorylation of c-Cbl Leads to the Interdependent Ubiquitination of Both Proteins. *J Biol Chem.* 276: 35185-35193, 2001

Reviewed and approved by site quality representative.

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